A New Lycopodine Alkaloid from *Phlegmariurus yunnanensis* CHING

by **Xiaoyan Xie**[a]), **Jinhe Jiang**[a]), **Ying Liu**[a]), **Kang Min**[b]), **Yongmei Xue**[a]), **Bi Jing**[a]), **Yan Zhang**[c]), and **Yegao Chen***[a])

[a]) Department of Chemistry, Yunnan Normal University, Street 121, Kunming 650092, P. R. China (phone and fax: +86-871-5516061; e-mail: ygchen48@gmail.com)

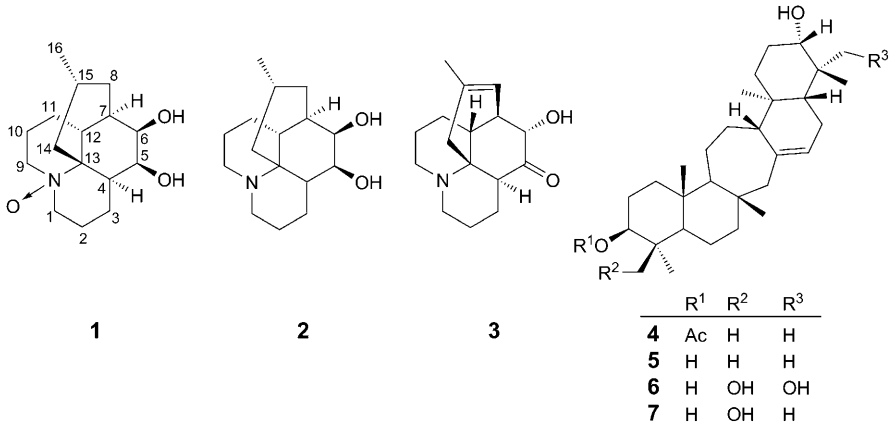
[b]) Diqing Institute of Agricultural Science, Shangrila 674400, P. R. China

[c]) Department of Chemistry, Kunming Medical College, West Reming Road, Kunming 650092, P. R. China

A new lycopodine alkaloid, 15α-methyllycopodane-5β,6β-diol *N*-oxide (**1**), was isolated from the whole plants of *Phlegmariurus yunnanensis* CHING, together with the two known alkaloids 15α-methyllycopodane-5β,6β-diol (**2**) and lycoposerramine H (**3**), and four serratene-type triterpenoids, serratenediol-3-acetate (**4**), serratenediol (**5**), lycocryptol (**6**), and serratriol (**7**). Their structures were elucidated on the basis of spectroscopic analyses, including HR-ESI-MS, ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY.

Introduction. – The Huperziaceae are comprised of two genera, *Huperzia* and *Phlegmariurus* [1]. Since huperzine A, a potent, reversible, and selective acetylcholinesterase inhibitor and a promising drug for the treatment of symptoms of *Alzheimer*'s disease, was discovered from *Huperzia serrata* (THUNB. ex MURRAY) TREV., numerous efforts on the isolation of new potent alkaloids from *H. serrata* and related plants have been carried out by many research groups, which led to the isolation of a large family of plant constituents, lycopodium alkaloids with diverse structures, including many unusual skeletons of interest from biogenetic and biological points of view and challenging targets for total synthesis [2–8]. *P. yunnanensis* CHING is an endemic species distributed in south-western parts of Yunnan, China. A previous investigation revealed the presence of the five alkaloids, huperzines A and B, lycodoline, lucidioline, and lycopodine (TLC evidence), but there is no report on the isolation of pure compounds [9]. As a part of our research on structurally unique and biologically active compounds from medicinal plants of Yunnan, China, we have isolated and identified a new alkaloid, 15α-methyllycopodane-5β,6β-diol *N*-oxide (**1**), as well as the two known alkaloids, 15α-methyllycopodane-5β,6β-diol (**2**) and lycoposerramine H (**3**), and the four serratene-type triterpenoids, serratenediol-3-acetate (**4**), serratenediol (**5**), lycocryptol (**6**), and serratriol (**7**) from *P. yunnanensis* [10–14]. This article focuses on the isolation and structural elucidation of compound **1**.

Results and Discussion. – 15α-Methyllycopodane-5β,6β-diol *N*-oxide (**1**) was obtained as a colorless amorphous powder. Its molecular formula was established as $C_{16}H_{27}NO_3$ by HR-ESI-MS analysis. The IR absorptions at 3421 and 3319 cm^{-1}



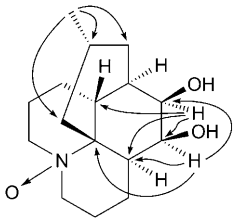
1 **2** **3**

	R^1	R^2	R^3
4	Ac	H	H
5	H	H	H
6	H	OH	OH
7	H	OH	H

suggested the presence of OH groups. In the EI-MS, the fragment peak at *m*/*z* 265 ($[M-16]^+$), due to the loss of an O-atom, suggested the presence of an *N*-oxide function [15]. The ^{13}C-NMR and DEPT spectra of **1** exhibited the characteristic peaks of lycopodine alkaloids, displaying 16 signals, including those for one Me, eight sp^3-CH_2, and six sp^3-CH groups, as well as for a sp^3 quaternary C-atom. The two signals for O-bearing CH groups at δ(C) 78.8 and 76.8 suggested the presence of two secondary OH groups. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Table*) with those of the known 15α-methyllycopodane-5β,6β-diol (**2**), which was also obtained in this study, indicated that the two compounds were similar, except for an additional *N*-oxide function in **1**. From further data, including a HMBC spectrum (*Fig. 1*), the structure of compound **1** was identified as a novel alkaloid, which was named 15α-methyllycopodane-5β,6β-diol *N*-oxide.

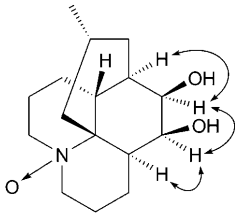
Table. *^{1}H- and ^{13}C-NMR Data* (CD_3OD, 500 and 125 MHz) *of Compound* **1**. δ in ppm, *J* in Hz.

	δ(C)	δ(H)
CH_2(1)	65.0 (*t*)	2.92 (*dd*, *J* = 3.5, 12.8), 3.58 (*dt*, *J* = 4.0, 13.0)
CH_2(2)	23.2 (*t*)	1.78–1.82 (*m*), 1.98–2.04 (*m*)
CH_2(3)	21.9 (*t*)	1.52–1.57 (*m*), 1.96–2.01 (*m*)
H–C(4)	35.4 (*d*)	2.79–2.85 (*m*)
H–C(5)	74.8 (*d*)	3.86 (*d*, *J* = 6.0)
H–C(6)	78.8 (*d*)	3.66 (*s*)
H–C(7)	44.8 (*d*)	1.93–1.97 (*m*)
CH_2(8)	40.7 (*t*)	1.17 (*dt*, *J* = 5.0, 13.0), 1.60–1.71 (*m*)
CH_2(9)	60.8 (*t*)	2.78 (*dd*, *J* = 2.7, 12.5), 4.23 (*dt*, *J* = 3.5, 12.0)
CH_2(10)	21.5 (*t*)	2.25–2.27 (*m*), 1.58–1.62 (*m*)
CH_2(11)	25.3 (*t*)	2.23–2.27 (*m*), 1.39–1.42 (*m*)
H–C(12)	39.3 (*d*)	2.11–2.16 (*m*)
C(13)	76.8 (*s*)	–
CH_2(14)	33.4 (*t*)	2.11–2.16 (*m*), 1.98–2.02 (*m*)
H–C(15)	25.1 (*d*)	2.88 (*d*, *J* = 6.5, 12.5)
Me(16)	24.1 (*q*)	0.89 (*d*, *J* = 6.5)



1 Fig. 1. *Key HMBCs* (H → C) *for* **1**

Three signals for N-bearing C-atoms at δ(C) 49.0, 49.1, and 66.1 (for C(1), C(9), and C(13), resp.) in **2** shifted downfield to δ(C) 65.0, 60.8, and 76.8, respectively, in **1** further confirmed the presence of an *N*-oxide group in **1** [16]. In the HMBC spectrum of **1**, the signal of H–C(6) correlated with the signals of C(4), C(5), and C(12), and the signal of H–C(5) correlated with the signals of C(4), C(6), C(7), and C(13), supporting the location of OH groups at C(6) and C(7). Correlations between the signals at δ(H) 2.79–2.85 (H–C(4)) and 3.86 (H–C(5)), and 3.86 (H–C(5) and H–C(6)), and at δ(H) 3.66 (H–C(6)) and 1.93–1.97 (H–C(7)) in the ROESY spectrum (*Fig. 2*) suggested that the OH groups at both C(5) and C(6) were β-oriented.



1 Fig. 2. *Major ROESY correlations for* **1**

This investigation was supported by a grant (No. 2005DFA30670) for international collaborative research by *Ministry of Science and Technology*, P. R. China, a grant (30560178) from the *Natural Science Foundation of China*, a program for *New Century Excellent Talents in University* (NCET-05-0824), and the *West Light Program* from the Chinese Academy of Sciences.

Experimental Part

General. Solvents were of industrial purity and distilled prior to use. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical Factory*, Qingdao, P. R. China), silica gel *H* (10–40 μm, *Qingdao Haiyang Chemical Factory*), *RP-18* (40–75 μm, *Fuji Chemical Industrial Co., Ltd.*, Tochigi, Japan), and *Sephadex LH-20* (25–100 μm, *Pharmacia Fine Chemical Co. Ltd.*). TLC: silica gel *GF254* (*Yantai Jiangyou Silica Gel Co. Ltd.*, Yantai, China). Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR: *Perkin-Elmer 241* polarimeter. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker DRX-AV-500* spectrometer; δ in ppm, *J* in Hz. MS: *Finnigan MAT 95* instrument and *VG Auto Spec-3000* spectrometer; in *m*/*z* (rel. int.).

Plant Material. The whole plants of *P. yunnanensis* were collected from Gongshan County of Yunnan Province, China in April, 2006, and identified by Prof. *Shugang Lu*, School of Life Science, Yunnan University, China, where a voucher specimen (No. 01232006) is deposited.

Extraction and Isolation. Air-dried powdered whole plants (419 g) were extracted with 95% EtOH (4×) at r.t., and the concentrated extract was adjusted to pH 2 with 1 mol/l H_2SO_4 soln. and then extracted with AcOEt. The acidic soln. was then basified to pH 10 with concentrated NH_4OH, and extracted with $CHCl_3$ and BuOH successfully. The BuOH extract (4.2 g) was subjected to CC (SiO_2; gradient $CHCl_3$/acetone): *Frs. 1–7. Fr. 5* (200 mg) was separated on CC (SiO_2; $CHCl_3$/MeOH 25:1 to 1:1; then *Sephadex LH-20*; $CHCl_3$/MeOH 3:2): **1** (10 mg) and **2** (20 mg). The $CHCl_3$ extract (2.1 g) was isolated on CC (SiO_2; gradient $CHCl_3$/MeOH): *Frs. 1–5. Fr. 3* (60 mg) was further subjected to CC (*RP-18*; H_2O/MeOH 3:7; then *Sephadex LH-20*; $CHCl_3$/MeOH 3:2): **3** (15 mg). The AcOEt extract (22 g) was subjected to CC (SiO_2; gradient $CHCl_3$/acetone): *Frs. 1–5. Fr. 1* (200 mg) and *Fr. 2* (150 mg) were crystallized, resp., from MeOH: **4** (20 mg) and **5** (15 mg). *Fr. 4* (300 mg) was purified on CC (SiO_2; $CHCl_3$/MeOH 50:1 to 1:1; then *Sephadex LH-20*; $CHCl_3$/MeOH 3:2): **6** (30 mg) and **7** (12 mg).

15α-Methyllycopodane-5β,6β-diol N-oxide (=(*1S,8a*R,*9*R,*11*R,*12a*R,*13*R,*14*S)-*Dodecahydro-11-methyl-1,9-ethanopyrido[2,1-*j*]quinoline-13,14-diol 5-Oxide*; **1**). Colorless amorphous powder. $[\alpha]_D^{25} = -1.43$ ($c = 0.00521$, MeOH). IR (KBr): 3421, 3319, 2934, 2365, 1631, 1461, 1113, 1030, 988. ^{1}H- and ^{13}C-NMR: see *Table*. EI-MS: 281 (2, M^+), 265 (17), 263 (46), 262 (72), 208 (100), 206 (42), 162 (26), 148 (25), 137 (13), 132 (7). HR-ESI-MS: 282.2073 ($[M+H]^+$, $C_{16}H_{28}NO_3^+$; calc. 282.2069).

REFERENCES

[1] Delectis Florae Reipublicae Popularis Sinicae Agendae, Academiae Sinicae Edita, 'Flora Reipublicae Popularis Sinicae', Science Press, Beijing, 2004, Vol. 6, p. 1.
[2] J.-S. Liu, Y.-L. Zhu, C.-M. Yu, Y.-Z. Zhou, Y.-Y. Han, F.-W. Wu, B.-F. Qi, *Can. J. Chem.* **1986**, *64*, 837.
[3] X.-C. Tang, Y.-F. Han, X.-P. Chen, X.-D. Zhu, *Acta Pharmacol. Sin.* **1986**, *7*, 501.
[4] K. Katakawa, N. Kogure, M. Kitajima, H. Takayama, *Helv. Chim. Acta* **2009**, *92*, 445.
[5] K. Katakawa, A. Nozoe, N. Kogure, M. Kitajima, M. Hosokawa, H. Takayama, *J. Nat. Prod.* **2007**, *70*, 1024.
[6] T. Kubota, H. Yahata, S. Yamamoto, S. Hayashi, T. Shibata, J. Kobayashi, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3577.
[7] Y. Hirasawa, T. Tanaka, K. Koyama, H. Morita, *Tetrahedron Lett.* **2009**, *50*, 4816.
[8] J. He, X.-Q. Chen, M.-M. Li, Y. Zhao, G. Xu, X. Cheng, L.-Y. Peng, M.-J. Xie, Y.-T. Zheng, Y.-P. Wang, Q.-S. Zhao, *Org. Lett.* **2009**, *11*, 1397.
[9] X.-Q. Ma, S.-H. Jiang, D.-Y. Zhu, *Biochem. Syst. Ecol.* **1998**, *26*, 723.
[10] H. Takayama, K. Katakawa, M. Kitajima, K. Yamaguchi, N. Aimi, *Chem. Pharm. Bull.* **2003**, *51*, 1163.
[11] N. Miller, C. Hootele, J. C. Braekman, *Phytochemistry* **1973**, *12*, 1759.
[12] H. Seto, K. Furihata, G. Xu, X. Cai, D. Pan, *Agric. Biol. Chem.* **1988**, *52*, 1797.
[13] Y. Tsuda, T. Fujimoto, K. Isobe, T. Sano, M. Kobayashi, *Yakugaku Zasshi* **1974**, *94*, 970.
[14] Y. Tsuda, K. Isobe, T. Sano, *Chem. Pharm. Bull.* **1975**, *23*, 264.
[15] C.-H. Tan, D.-Y. Zhu, *Helv. Chim. Acta* **2004**, *87*, 1963.
[16] X.-T. Tong, C.-H. Tang, X.-Q. Ma, B.-D. Wang, S.-H. Jiang, D.-Y. Zhu, *Planta Med.* **2003**, *69*, 576.

Received November 23, 2009